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Effect of Hemorrhagic Shock on Cefazolin and Gentamicin Pharmacokinetics in Dogs

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The physiologic response to traumatic injury may alter the disposition of drugs and thereby affect their therapeutic or toxic potential. A study was conducted in 10 mongrel dogs to determine the effect of experimental hemorrhagic shock with resuscitation on the pharmacokinetics of gentamicin and cefazolin. Single simultaneous intravenous doses of gentamicin (3 mg/kg) and cefazolin (25 mg/kg) were administered to each animal on an initial study day, after which serial blood and urine collections were performed. After 1 week, a standard hemorrhagic shock model was applied to each animal. Shock was continued for 1 h, after which the animal was resuscitated with either whole blood or saline. After stabilization for 20 min, a second dose of gentamicin and cefazolin was administered, and blood and urine were again collected. Drug clearance was not significantly altered, except for that of cefazolin after saline resuscitation, for which there was a significant increase in drug clearance. After both methods of resuscitation an increase in the volume of distribution was noted for cefazolin and gentamicin. Drug half-life was noted to be increased after shock for cefazolin by both resuscitation methods and for gentamicin after shock by saline resuscitation. Although alterations of pharmacokinetic parameters were noted, mean concentrations of gentamicin and cefazolin in serum were similar for pre- and postshock phases.

The physiologic response to traumatic injury may impose alterations in the disposition of drugs that may affect their therapeutic or toxic potential. A common result of traumatic injury is hemorrhage, which may proceed to shock. With hemorrhagic shock, immediate changes in cardiovascular parameters have been well documented and include decrease in cardiac output, blood volume, increase in systemic vascular resistance and redistribution of blood flow (6). These changes then cause an alteration in the blood flow to major organs. Blood flow to heart, liver, and brain is maintained at the expense of the kidneys, gut, and skin (6). This latter effect is mediated by the sympathetic, mineralocorticoid, and antidiuretic hormone responses (1). A decrease in blood volume and renal blood flow also stimulates the antidiuretic hormone, resulting in lowered urine output.

As a result of these responses to hemorrhagic shock, alterations in drug disposition relative to that in nonshock states may occur. The disposition of drugs that are eliminated primarily by renal excretion is likely to be affected. Concentrations of renally eliminated agents in serum may be increased when given after hemorrhagic shock compared with those in the absence of shock. Therefore, traditional doses of renally eliminated drugs may prove excessive in the face of hemorrhagic shock and may present a greater potential for toxicity.

After traumatic injury, in which hemorrhage and shock are frequently present, antimicrobial agents are often necessary to prevent the development of subsequent infection. At present, little information is available to suggest proper dosage schedules of antimicrobial agents after hemorrhagic shock and fluid resuscitation, particularly for renally excreted agents. Our intention was to examine the effect of hemorrhagic shock with resuscitation on the disposition (pharmacokinetics) of two commonly used antimicrobial agents, cefazolin and gentamicin. These agents were chosen for study because they are representatives of classes of antimicrobial agents (β-lactams and aminoglycosides) that are commonly used after traumatic injuries with bacterial comtamination. Cephalosporins such as cefazolin are active against most gram-positive bacteria, while most gram-negative bacteria are susceptible to aminoglycosides.

MATERIALS AND METHODS

Study design. The pharmacokinetic profiles of cefazolin sodium and gentamicin sulfate were determined in 10 adult mongrel dogs after a single intravenous dose. Each animal was studied on 2 days separated by 1 week. On study day 1 the dogs were treated under the conditions described below, in the absence of hemorrhagic shock; and on day 2 the dogs were treated following resuscitation from hemorrhagic shock. Each animal served as its own control. The study was approved by the Animal Use Review Committee at the Medical College of Georgia.

On each study day the animals were anesthetized with sodium pentobarbital, intubated, and placed in a supine position. Ventilatory assistance was begun if the respiratory rate was less than 5 respirations per minute. Intravenous and intraarterial catheters were placed for continuous monitoring of arterial pressure, blood sampling, and fluid administration. All urine was collected on each study day through a urethral catheter. Continuous monitoring of the electrocardiogram and arterial pressure (systolic, diastolic, and mean) was performed with an oscilloscope and monitor (models

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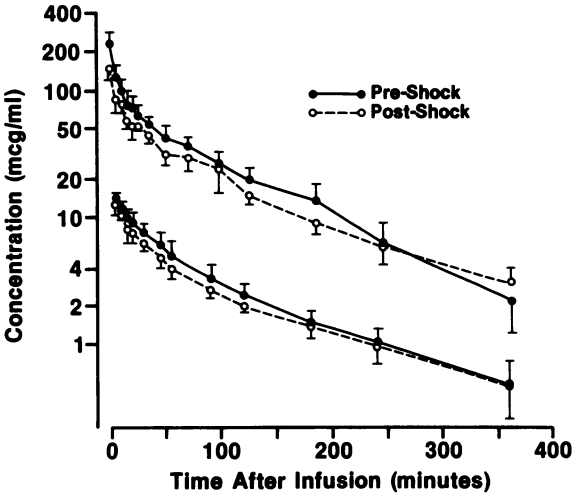

FIG. 1. Mean concentration (with standard deviations) of cefazolin (upper curves) and gentamicin (lower circles) in serum prior to hemorrhagic shock (●) and after hemorrhagic shock with whole blood resuscitation (○).

78304A, 78205B, and 78213C; Hewlett-Packard Co., Palo Alto, Calif.).

After initial stabilization of each animal, base-line laboratory values including hematocrit, serum creatinine, total protein, and albumin were determined. On each study day cefazolin sodium (25 mg/kg) immediately followed by gentamicin sulfate (3 mg/kg) were each administered intravenously over 5 min. In previous studies it has been demonstrated that cephalosporins (particularly cefazolin) do not result in significant aminoglycoside inactivation (4, 7, 8). Arterial blood samples were collected just before drug administration and at 0, 5, 10, 15, 20, 30, and 45 min and 1, 1.5, 2.0, 2.5, 3.0, 4.0, and 6 h after gentamicin administration was complete. Serum was separated and frozen at −25°C until time of analysis. All urine was collected in 30-min intervals for a total of 4 h, followed by two collections at 1-h intervals. The volume from each collection was recorded, and a fraction was frozen until analysis. At the completion of the sampling period on day 1, the animals were allowed to recover for 7 days before study day 2.

On study day 2, the animals were prepared as described for study day 1. Large-bore (14-gauge) catheters were placed in the left external jugular and common femoral veins. In addition, a 14-gauge catheter was placed in the common femoral artery and connected to the pressure-monitoring aparatus. After 20 min of stabilization, each animal was heparinized with 100 U of sodium heparin per kg of body weight. By using a modification of the Wigger and Werle (10) hemorrhagic shock model, hemorrhage was induced by blood removal from the arterial catheter over a period of 15 to 20 min, until a mean arterial pressure of 50 mm Hg was obtained. Hemorrhage to a mean arterial pressure of 50 mm Hg was adequate to demonstrate significant shock, as well as survivability after resuscitation (9–11). This pressure was maintained for a period of exactly 1 h. At the end of this 1 h the animals were resuscitated by intravenous administration of fluids over 5 to 10 min.

The animals were divided into two groups to study resuscitation techniques. Five dogs received fluid resuscitation with 0.9% sodium chloride in a volume equal to three times that of the blood removed (5). The remaining five animals were resuscitated with 1 liter of 0.9% sodium chloride and the entire volume of the previously withdrawn heparinized whole blood. After a 20-min stabilization period, the antibiotics were administered as described above with the same serum and urine sampling procedures. At the end of the study, each animal was sacrificed.

Drug analysis. Cefazolin in serum and urine was assayed by a reverse phase, high-performance liquid chromatographic technique (S. M. Bayoumi, J. J. Vallner, and J. T. DiPiro, Int. J. Pharm. in press) with a pump (model 590; Waters Associates, Inc., Milford, Mass.) with autosampler (WISP), a UV detector (481 Lambdamax; Waters Associates), and a recorder-integrator (model 3390A; Hewlett-Packard). A column (15 cm; C-18; Waters Associates) was used with a mobile phase consisting of acetonitrile and 0.1 M acetate buffer (pH 3.85) in a 11:89 ratio and with sulfamethoxazole serving as an internal standard. In previous studies the sensitivity of this assay was 0.5 μg/ml, with coefficients of variation (within-day) of 2.5 and 3.12% at 2 and 20 μg/ml, respectively.

Gentamicin in serum was assayed by a fluorescence polarization immunoassay (3), by using a fluorescence polarizer analyzer (TDX; Abbott Laboratories, Chicago, Ill.). The technique allowed detection of gentamicin from 0.25 to 12 μg/ml. Coefficients of variation (within-day) determined at our institution over the 2- to 10-μg/ml range were 2 to 3.5%. Investigators have demonstrated previously (3) the specificity of this method and have shown negligible interference by other aminoglycosides and a wide range of commonly used drugs. A standard curve was prepared by using six concentrations, between 0.25 and 12 μg/ml.

Pharmacokinetic analysis. Pharmacokinetic parameters were calculated for each animal on each day by using noncompartmental analysis. Specifically, total clearance (CL) and volume of distribution at steady state (V_{ss}) were calculated for each drug, as was the terminal elimination rate constant (k_{el}) and half-life ($t_{1/2}$) (2). Renal clearance (CL_R) was calculated for cefazolin only because this agent is known to have dual pathways of elimination that may be altered by the model.

The areas under cefazolin and gentamicin serum concentration versus time curves (AUCs) from 0 to 6 h after drug

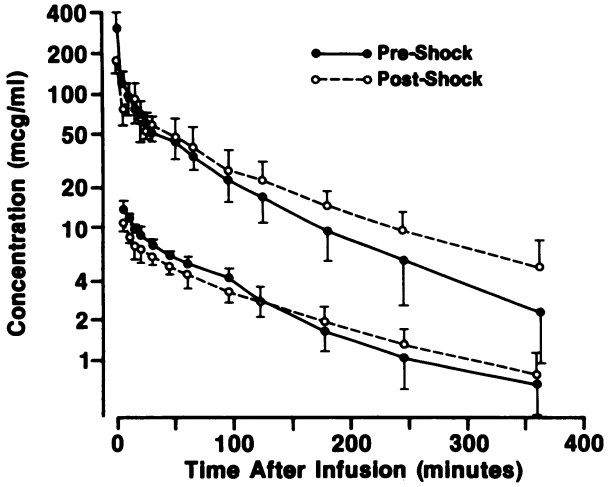

FIG. 2. Mean concentrations (with standard deviations) of cefazolin (upper curves) and gentamicin (lower curves) in serum prior to hemorrhagic shock (●) and after hemorrhagic shock with saline resuscitation (○).

TABLE 1. Mean pharmacokinetic parameters by method of resuscitation of cefazolin and gentamicin

Drug and parameter	Mean ± SD for pharmacokinetic parameters			
	Preshock		Postshock	
	Saline	Blood	Saline	Blood
Cefazolin				
$t_{1/2}$ (min)	85.6 ± 15.2	73.8 ± 14.6[a]	108.6 ± 28.9	96.3 ± 13.2
CL (ml/min per kg)	3.38 ± 1.05[a]	3.13 ± 0.76	2.75 ± 1.10	3.59 ± 0.78
CL_R (ml/min per kg)	2.60 ± 0.56	2.61 ± 0.51	1.77 ± 0.75	2.52 ± 0.70
V_{ss} (liter/kg)	0.286 ± 0.059	0.278 ± 0.063	0.329 ± 0.12	0.403 ± 0.056
V_{area} (liter/kg)	0.410 ± 0.094	0.332 ± 0.089[a]	0.427 ± 0.174	0.499 ± 0.098
Gentamicin				
$t_{1/2}$ (min)	96.1 ± 31.7	106.8 ± 36.0	132.3 ± 39.3	107.5 ± 33.6
CL (ml/min per kg)	2.51 ± 0.41	2.88 ± 0.628	2.64 ± 0.75	3.28 ± 0.55
V_{ss} (liter/kg)	0.264 ± 0.039[a]	0.329 ± 0.067[a]	0.404 ± 0.120	0.370 ± 0.110
V_{area} (liter/kg)	0.351 ± 0.081[a]	0.433 ± 0.124	0.450 ± 0.122	0.490 ± 0.093

[a] Indicates significant difference ($P < 0.05$) between pre- and postshock groups by paired t test.

administration were calculated by using a logarithmic trapezoidal method and were corrected for the infusion time. The AUC from 6 h to infinity after drug administration was estimated by dividing the concentration at 6 h by k_{el}. The area under the curve of the product of concentration and time versus time (area under the moment curve; AUMC) from 0 to 6 h after drug administration was calculated by the linear trapezoid method. The AUMC from 6 h to infinity after drug administration was estimated from the 6-h concentration-time product divided by k_{el} and then added to the 6-h concentration divided by k_{el}^2.

The k_{el} was calculated from the slope of the natural log of serum concentration versus time plot from 2 to 6 h (log-linear portion). The terminal $t_{1/2}$ was defined as $t_{1/2} = 0.693/k_{el}$. CL was determined by dividing the dose administered by the AUC. The volume of the distribution by area (V_{area}) was calculated by dividing the dose administered by the product of AUC and k_{el}.

The CL_R was calculated for cefazolin as the total amount of drug excreted in the urine in 6 h divided by the AUC for drug in serum from 0 to 6 h. V_{ss} was calculated from the following relationship: V_{ss} = (dose × AUMC/AUC^2) − (dose × 5 min/2× AUC). This was expressed as liters per kilogram of body weight (2).

Statistical analysis. Mean values for each parameter were calculated for the animals in the saline and whole blood resuscitation groups for each study day. Differences between pre- and postshock data were compared by the paired t test. A P value of 0.05 was accepted as significant.

RESULTS

A total of 13 animals were entered into the trial, and 10 animals completed (6 male and 4 female) the trial. Of the three deaths, two animals expired during the shock phase of the study day 2, while one animal expired for unknown reasons after the study day 1 was completed. The 10 animals that completed the trial weighed a mean of 15.2 ± 3.9 kg (range, 9.1 to 20.5 kg) and had normal values for the laboratory screen tests.

To produce a mean arterial pressure of 50 mm Hg, a mean of 634 ml of blood was withdrawn (range, 240 to 1180 ml) which represented 25 to 59 ml/kg and was estimated to be about 50% of the total blood volume. In several animals additional blood (10 to 40 ml) was withdrawn to maintain mean arterial pressure at 50 mm Hg for the specified time. The preshock mean arterial pressure ranged from 105 to 120 mm Hg, which was brought to 50 mm Hg during the shock phase and subsequently returned during the postshock phase to 85 to 95 mm Hg in all animals.

Urine output averaged 2.8 ml/h during the shock phase and then 54 ± 13 and 157 ± 65 ml/h during postshock for saline and whole blood resuscitation, respectively ($P < 0.01$). Hematocrit was a mean of 34% in the preshock phase and then 11.7 and 25% after saline and whole blood resuscitation, respectively.

Pharmacokinetics. Mean concentrations of cefazolin and gentamicin in serum are presented for animals that received whole blood (Fig. 1) and saline (Fig. 2) resuscitation. Concentrations of each drug in serum after hemorrhagic shock by both resuscitation methods were similar to those before shock.

Mean pharmacokinetic parameters are given in Table 1. For most comparisons, significant differences were not noted. The mean cefazolin $t_{1/2}$ was significantly higher postshock (30% increase) after whole blood resuscitation. With saline resuscitation, cefazolin $t_{1/2}$ was increased 26% postshock, but this difference was only significant at $P < 0.1$. Because there were few observations, the probability of a type II error is relatively high. Cefazolin CL was noted to be significantly lower postshock with saline resuscitation; however, it was increased postshock with whole blood resuscitation (nonsignificant). For both saline and whole blood resuscitation, the mean V_{ss} increased postshock (14 and 43%, respectively), but these differences were not significant. However, V_{area} was significantly increased postshock with whole blood resuscitation (not significant with saline resuscitation).

For gentamicin, the $t_{1/2}$ was significantly longer postshock after saline resuscitation (increase of 38%) but not with whole blood resuscitation. By both resuscitation methods the gentamicin CL during pre- and postshock was not significantly different, while V_{ss} was significantly increased (53 and 19%, respectively, with saline and whole blood resuscitation). V_{area} was significantly increased after saline resuscitation but not with whole blood resuscitation.

DISCUSSION

This model was designed to determine if hemorrhagic shock and fluid resuscitation alters the pharmacokinetics of two renally excreted antimicrobial agents (cefazolin and

gentamicin). Overall, dramatic differences in concentrations in serum pre- and postshock for saline and whole blood resuscitation were not observed. Subtle differences in pharmacokinetic parameters were observed.

For both resuscitation methods and with both agents studied, increases in V_{ss} and V_{area} were noted. For these agents, the V_{ss} was related to the extracellular fluid volume. This model therefore indicates that there is an increase in the extracellular fluid volume after hemorrhagic shock and fluid resusitation.

Cefazolin and gentamicin CLs were similar during pre- and postshock, with the exception of cefazolin after whole blood resuscitation, in which the CL postshock was significantly reduced (19%). Because these agents are primarily excreted renally, our results indicate that renal drug excretory capability is promptly restored by immediate fluid resuscitation after hemorrhagic shock of relatively short duration. With continued hemorrhage or in those cases in which hemorrhage is accompanied by significant tissue or organ trauma, the pharmacokinetics of cefazolin and gentamicin could still be significantly affected. The information obtained from the results of this study suggests that in humans in which significant blood loss occurs but in which fluid is replaced promptly and in which there is minor tissue injury, dosage regimens of cefazolin and gentamicin should approximate those required in patients who have not had significant hemmorrhage.

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